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(54) METHOD OF MAKING REAGENT TEST DEVICE AND REAGENT TEST DEVICE MADE ACCORDING TO THIS METHOD

(71) We, ALFA-LAVAL AB, a Swedish Corporate Body of Postfack, S-147 00 Tumba, Sweden,

do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a reagent test device, comprising a carrier and at least two substances, supported by said carrier, to be activated upon use of the reagent test device.

Reagent test devices as mentioned above have hitherto been made in several different ways. In one known method, one of the substances is encapsulated in so called microcapsules that are suspended in a liquid containing the other substance, whereupon the microcapsules and the liquid are applied to a carrier in one way or other. This method of manufacture is rather expensive.

In another known method, a reagent test device is made by impregnating a carrier with a porous structure in two separate zones, with liquids containing the substances. This method of manufacture is complicated due to the difficulties in impregnating one and the same carrier with two different liquids.

According to the present invention there is provided a method of making a reagent test device including a carrier and at least two reaction substances supported by said carrier, the method comprising so applying by printing at least two liquids, each of which contains one of said substances, directly to one surface of the carrier that said substances will remain on the surface separated by a predetermined interspace.

The above method is simple and cheap, and can result in reagent test devices suitable for making a quantitative analysis of high accuracy.

The invention also provides a reagent test device, comprising a carrier and at least two reaction substances supported on the same surface of said carrier, and separated from each other by a predetermined interspace.

Preferably at least one of said substances is applied to the carrier in such a way, that it remains fixed to it even upon use of the reagent test device.

In accordance with one preferred method the substances in question are applied to said surface of the carrier at a plurality of locations, spaced apart with small interspaces. For example, the substances can be applied as dots and/or stripes on the surface. The locations may be mixed on the surface.

The method of the invention can be used for making several reagent test or indicator systems, for example as disclosed in U.S. Patent Specifications 3,092,463, 3,511,608, 3,549,328 and 3,926,732.

As mentioned above, the substances can be applied to a plurality of locations on the surface of the carrier with very small interspaces between them. This does not means, however, that the scope of the invention is limited to cases where interspaces of microscopic orders are necessary. Even in such cases, where the distance between the substances shall be somewhat larger, e.g. about 1 mm, the invention applies, as an interaction between the different substances on the surface of a reagent test device carrier is still possible where the interspace between the substances is of this size, if a liquid that is to be brought into contact with the reagent test device carrier has to be able to penetrate through the substances to provide a diffusion means, for example for carrying part of one of the substances to the other substance. In such a case it is obvious that utmost accuracy for example to one or a few hundredths of a mm is needed

regarding the interspace between said substances.

It has been found, that a printed text or picture can consist of a plurality of minute little dots situated at microscopic distances from each other, which distances cannot normally be detected by the naked eye, and a printed colour picture which the eye perceives as just a single colour, may in fact consist of a plurality of dots of different colours. Thus a picture which the eye perceives as being green can consists of a plurality of blue and yellow dots.

Using conventional printing techniques two or more reagent substances can be applied to the surface of a reagent test carrier with the interspace between the substances being accurately predetermined.

Thus in making the reagent test device the substances are applied to the carrier using printing techniques, known per se, for example photogravure printing, in which the substances, dissolved in appropriate solvents, are applied to the surface of the carrier by printing rollers having very small depressions or pores of different depths. Alternatively, silk screening can be used, in which each substance, dissolved in an appropriate solvent, is pressed out through a line screen mesh placed around a rotatable roller.

There are several types of printing techniques, known per se, which are not generally called "conventional". There are for example different kinds of plateless printing techniques, like direct electrostatic printing, indirect electrostatic printing and ink-jet printing, which have gained more and more importance in more recent years, and which can be used for making reagent test devices.

In direct electrostatic printing electrostatic charges are created on specially coated paper which has a conducting layer covered by an insulating layer. The electrostatic charge is developed into visible image by a toner, which can be a liquid containing the desired reagent substance.

Indirect electrostatic printing is an offset process where the electrostatic charge is held on an intermediate surface (such as a drum) and only the toner, containing the desired reagent substance, is transferred and fixed to the paper. This method is used in the Xerox® copying system.

Ink-jet printing has developed very fast lately. There are many different systems, but they all depend on continuous or discontinuous flows of very thin liquid jets, that are directed with great accuracy in the desired direction toward the carrier in question.

Any type of reproducing graphic technique may be used to perform the present invention.

When substances are applied to a plurality of locations with very small interspaces, it should be noted, that there is an advantage that a colour change, due to a reaction of the substances when the reagent test device is being used, will be perceived as a simultaneous colour change over a relatively large area. (The surrounding parts of the reagent test device carrier preferably have the same colour as said surface had before any colour change). Such reagent test device for example comprising two substances, will give a more reliable indication of a positive reaction than a reagent test device having one surface, covered with one of the substances only, which changes its colour gradually from one part to another part, as the second substance is diffusing along the surface. Thus, if said substance is completely consumed before it has diffused over the entire surface area, whereby a colour change will occur in just part of the surface area, there may arise doubt as to the reliability of the result indicated by the reagent test device. The possibility of using a reagent test device for quantitative analysis is also improved if the colour change occurs over a relatively large surface area.

A better understanding of the present invention will now be had from the following detailed description given by way of example with reference to the accompanying drawing, in which:—

Figures 1 to 4 show schematically different reagent test devices made according to the invention;

Figure 5 illustrates schematically the preparation of a test device in accordance with Figures 1 to 4.

Referring to Figures 1 to 4, three different reagent test devices are shown, for example intended for indication of the presence of a certain enzyme in a liquid. The reagent test devices comprise carriers 1, 2, 3 and 4. Two reagent substances A, B have been printed with conventional printing technique in various patterns on these carriers. A reagent test device of this kind may be intended for dipping into a sample of said liquid, removing it from same to let a thin liquid layer remain on the carrier. One of the substances on the carrier, for example substance A, may then be brought to diffuse through the thin liquid layer, toward the other substance, i.e. substance B. The enzyme to be indicated in the liquid may react with substance A, or catalyze a chemical reaction caused by substance A. Thus substance A is completely or partly consumed during its migration toward substance B dependent

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on the concentration of the enzyme in the liquid. If substance A is completely consumed by the enzyme in the liquid, no reaction can occur between substances A and B. If part of substance A reaches substance B, these will react, substance A and B being of such a nature, that a colour change will occur and over the total surface area, onto which substances A and B are applied. The intensity of the colour change is dependent on the concentration of the enzyme to be quantitatively estimated in the liquid. Substances A and B of course can interact in other ways. For example, they may react with each other in a first stage, giving rise to an intermediate substance without any colour change. Then in a second stage this intermediate compound may react with the enzyme of interest to bring about a colour change. Alternatively, there may occur a first colour change as the intermediate substance is formed, and a second colour change when the intermediate substance reacts with the enzyme. Such a system would make it possible to decide safely, if a reagent test device has already been used, even if the enzyme reaction test had been completely negative.

In Figure 4 a reagent test device for the indication of any catalase enzyme present in a liquid is shown. The reagent test device comprises a carrier 4 and substances A, B and C applied to it using a conventional printing technique.

Substance A contains a peroxidase enzyme and a dye, for example o-tolidine. Substance B contains the enzyme glucose oxidase and substance C glucose. Enzymes peroxidase and glucose oxidase are both chemically fixed to cellulose particles, which are fixed to the carrier by means of any suitable binding agent using a printing technique. After the application of substances A and B the enzymes are immobile in relationship to each other and to carrier 4.

The reagent test device just described will operate in the following way when brought into contact with liquid to be tested.

1) The glucose in substance C is dissolved by the liquid and is spread over carrier 4.
2) In the vicinity of substance B enzyme glucose oxidase catalyses the reaction between glucose and oxygen, whereby hydrogen peroxide is obtained as a reaction product.
3) The hydrogen peroxide diffuses through the liquid provided in the areas between substances A and B, to the locations of substance A.
4) At the locations of substance A the dye o-tolidine is oxidized by hydrogen peroxide in the presence of enzyme peroxidase, and a blue colour is obtained.
5) If there is any enzyme catalase present in the liquid, the hydrogen peroxide is decomposed completely or partly. Catalase in a high concentration in the liquid will decompose the hydrogen peroxide completely before it has had time to diffuse to the locations of substance A, and no blue colour is obtained. A small concentration of catalase will decompose the hydrogen peroxide partly, and part of the hydrogen peroxide will diffuse to the locations of substance A to cause a blue colour. Thus, due to the concentration of enzyme catalase in the liquid a more or less intensive colour change is obtained on the reagent test device.

The reagent test devices shown in Figures 1 to 4 are prepared by printing techniques, as mentioned above. Reference is now made to Figure 5, which shows the principle of conventional printing. A sheet 5 is fed in the direction of the arrow. With two printing rollers 6 and 7 two different liquids, containing substances A and B, are printed on the sheet. As already mentioned there are many types of rollers, well known to those versed in the art, so no detailed description is needed.

Of course it is necessary to adjust the viscosity of the liquids, containing the substances in question, dependent on the printing technique chosen. To illustrate the invention further one specific example will now be described.

Example

10 grams of particulate CMC (carboxi methyl cellulose) were activated in the way well known to those skilled in the art.

Glucose oxidase (GO) (Boehringer) and peroxidase (PO) (Sigma Co) were immobilized on different samples of the activated CMC, also according to well known methods. The mixtures for printing were prepared in the following way:

Glucose oxidase/CMC

2.5 grams of wet GO/CMC were stirred in 20 mls of distilled water with the aid of a magnetic stirrer. 0.085 grams of colloidal CMC were added to adjust the viscosity of the mixture.

Peroxidase/CMC

2.5 grams of wet PO/CMC were stirred in 20 mls of distilled water with the aid of a magnetic stirrer. 0.085 grams of colloidal CMC were added to adjust the viscosity of the mixture. 0.033 grams of o-tolidine were added by stirring to the mixture.

The two mixtures thus obtained were printed by silk screening as distinct parallel lines, according to the system GO-PO-GO-PO etc, on a filter paper, that had been immersed in a 10% glucose solution in water and had been dried at 35°C.

WHAT WE CLAIM IS:—

1. A method of making a reagent test device including a carrier and at least two reaction substances supported by said carrier, the method comprising so applying by printing at least two liquids, each of which contains one of said substances, directly to one surface of the carrier that said substances will remain on the surface separated by a predetermined interspace.

2. A method according to Claim 1 wherein the said substances are applied to the surface of the carrier at a plurality of locations with small interspaces between them.

3. A method according to Claim 2, wherein the locations at which the at least two liquids are applied to the surface are mixed on said surface.

4. A method according to Claim 2 or 3, wherein at least one of the substances is applied as dots on said carrier surface.

5. A method according to Claim 2 or 3, wherein at least one of the substances is applied as stripes on said carrier surface.

6. A method according to any of Claims 1 to 5, wherein the substances are applied by photogravure printing.

7. A method according to any of Claims 1 to 5, wherein the substances are applied to the carrier surface by silk-screening.

8. A method according to any of Claims 1 to 5, wherein the substances are applied to the carrier surface by ink-jet printing.

9. A reagent test device, comprising a carrier and at least two reaction substances supported on the same surface of said carrier, and separated from each other by a predetermined interspace.

10. A reagent test device according to Claim 9, wherein the substances are applied to said surface at a plurality of locations with small interspaces between them.

11. A reagent test device according to Claim 10, wherein the said locations are mixed on said surface.

12. A reagent test device according to Claim 9 or 10, wherein the said substances are applied to said surface as a plurality of dots.

13. A reagent test device according to Claim 10 or 11, wherein the said substances are applied to said surface as a plurality of stripes.

14. A method according to Claim 1 and substantially as herein described.

15. A reagent test device substantially as herein described with reference to the accompanying drawings.

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1526708 COMPLETE SPECIFICATION

1 SHEET *This drawing is a reproduction of the Original on a reduced scale*

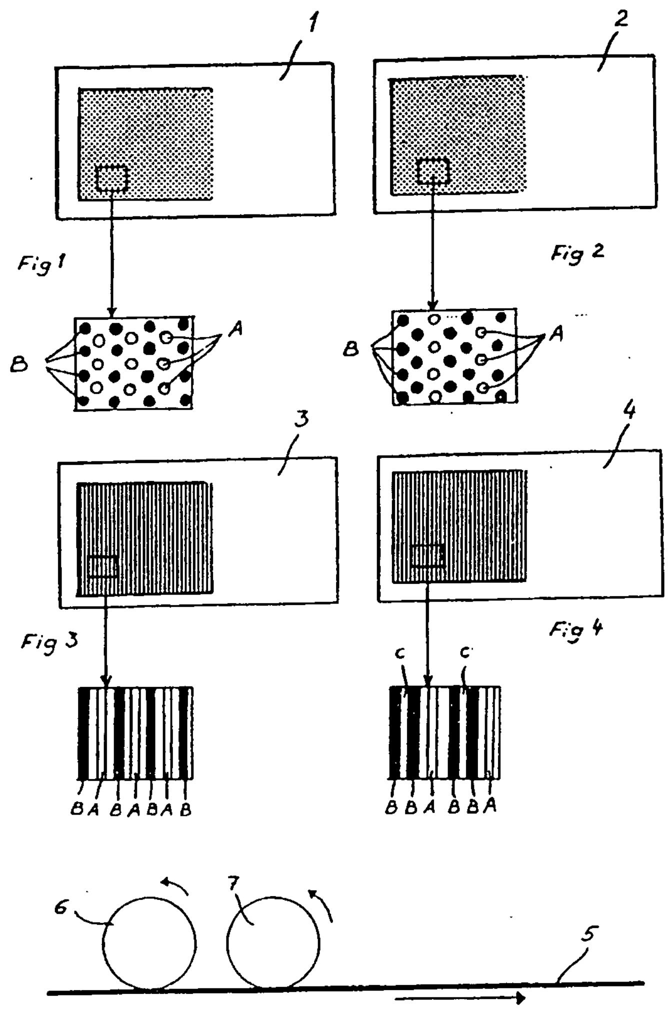

Fig 1

Fig 2

Fig 3

Fig 4

Fig 5